Standardizing methods and procedures for mouse retinal flat mounts and glial cell counts

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Introduction: The mammalian retina contains neuronal cells as well as a number of non-neuronal glial cells. The different types of glial cells include Müller glia, retinal astrocytes, and microglia. Müller glial cells and astrocytes nourish neurons and microglia act as sentinels that respond to injury or disease within the nervous system. The long-term goal of our laboratory has been to study interactions between microglia, Muller glia and astrocytes in healthy and diseased tissue. The focus of the present study was to develop a technique that would allow the laboratory to study changes in cell number in retinal flat mounts and cultures. Methods: Immunohistochemistry (IHC) was performed to fluorescently label mature murine retinal tissue. Retinal flat-mounts were stained with SOX2, a nuclear marker for glial cells or IBA1 for microglial cells, and counter-stained with Hoechst solution to label all nuclei. Pure cultures of mouse microglial cells treated with liposomal clodronate (a drug which specifically targets and ablates microglia) and vehicle were counter stained with Hoechst solution. Cell counts were performed on the images of the fluorescently labeled samples using Image-J software. **Results:** Convolutions were used to filter images of immunolabeled cultures and retinal flat mounts to make the images clear enough to capture cell number. The cell count assistance protocol yielded acceptable cell count results of the stained cells and determined a detectable difference in the number of clodronate treated cells versus vehicle treated control cells. The images produced of the retinal flat-mounts were analyzed to determine the percentage of SOX2 positive Müller glia in the mature murine retinal tissue. Conclusion: A modified Image J program could be used to determine cellular number in cultures and retinal flat mounts.

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